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Distal femoral intercondylar notch dimensions and their relationship to composition and metabolism of the canine anterior cruciate ligament

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Summary

Objective: To determine the relationship between the dimensions of the distal femoral intercondylar notch (ICN) and the composition and metabolism of the anterior cruciate ligament (ACL) in three dog breeds with different relevant risks to ligament rupture and subsequent osteoarthritis (OA).

Design: ICN measurements were obtained from the femurs of Golden Retrievers (high risk), Labrador Retrievers (high risk) and Greyhounds (low risk). Femoral condyle width and height, ICN height and width indices, and notch shape index were measured using Vernier callipers in all dogs. Intact ACLs were obtained from the same dog breeds for a study of the impinged areas and were analysed for collagen content, collagen cross-links, and sulphated glycosaminoglycan (GAG) content, matrix metalloproteinase (MMP)-2 and the tissue inhibitors of metalloproteinases (TIMPs)-1 and -2.

Results: Femoral condyle width and height and ICN width indices were significantly greater in the low risk compared to the high risk breeds ($P < 0.01$ for all parameters). In contrast, the pro ($P = 0.003$) and active ($P = 0.007$) forms of MMP-2 and sulphated GAGs ($P = 0.0002$) were significantly greater in the impinged areas of the ACLs of the rupture predisposed breeds.

Conclusions: Impingement by the ICN on the ACLs of the high risk breeds may result in increased collagen remodelling and increased sulphated GAG deposition, causing reduced structural integrity of the ligament. Altered ACL composition may predispose the ligament to increased laxity leading to joint degeneration and OA. This may have a comparative implication for pathogenesis of ACL rupture in humans.

Key words: Canine, Intercondylar notch, Ligament, Collagen, Glycosaminoglycans.

Introduction

The distal femoral intercondylar notch (ICN) is normally filled by the anterior cruciate ligament (ACL), posterior cruciate ligament and fat[1]. Stenotic or narrowed ICNs have been implicated as a risk factor in human ACL injuries[2,3]. A narrow ICN is due to increased osteophyte formation and is associated with both ACL rupture and osteoarthritis (OA) in dogs[4,5]. The ACL passing through a narrow ICN could impede the normal function of the ACL by impingement, and result in damage and joint laxity[6]. Knee laxity may itself contribute to ICN narrowing resulting in further ACL impingement and damage[5]. Young patients with congenital stenosis who sustained an ACL tear, by a non-contact injury, were found to have small ICN width indices[7]. In addition, young females incur injuries to the ACL more frequently than males, and this is associated with narrow ICNs[8].

Tendons and ligaments (T/L) that pass through or around bony structures in the mammalian skeleton are subject to compressive as well as normal tensile forces[9] and this is reflected in changes to their biochemical composition. For example T/Ls have increased proteoglycan content and become more fibrocartilaginous in compressed regions compared to tensile regions[10]. This can also be seen, histologically, at insertion sites of T/Ls to bone or where the T/Ls pass around a bone (e.g., flexor digitorum profundus tendon)[11]. In several abnormal conformational circumstances e.g., "impingement syndrome", ACL graft placement or ICN narrowing, bony impingement on the T/L is also increased. This may contribute to T/L damage, as seen in the case of ACL graft placement where impingement by the distal femoral ICN contributes to graft failure[12]. To date, it is not known if alterations in biochemical properties of the collagen fibres in ligaments at impinged/compressed sites in other species are adaptive or degenerative.

In dogs, a stenotic ICN causes impingement on the ACL, from either the medial aspect of the lateral femoral condyle or the intercondylar roof of the notch[4]. Other risk factors involved in canine ACL degeneration include ageing, bodyweight and immobilization[13,14]. Certain dog breeds such

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as the Labrador Retriever and, to a lesser extent, the Golden Retriever are at high risk for non-contact ACL rupture whereas other breeds such as the Greyhound are at low risk for ligament failure[15]. Differences in the ICN width and its influence on the biochemical composition of the ACL, in other species, have not been investigated to date. We report here a study performed in radiologically and anatomically normal dogs from breeds with a widely differing relative risk for ACL rupture. Stenotic ICNs in normal predisposed dogs may alter ACL composition thereby contributing to a reduced mechanical strength and increased likelihood of eventual rupture. Thus, by examining normal knees of dog breeds with divergent predisposition to ACL failure, we can investigate the intrinsic features of the ACL and NWI (notch width index) that might predispose to non-contact failure. Such a comparative approach may also provide a valuable insight into etiological factors influencing human non-contact ACL failure, a condition that invariably leads to OA[16].

Method

SAMPLE POPULATION

All dogs were euthanized for reasons other than musculoskeletal disease following approved guidelines from the Department of Clinical Veterinary Science regarding use of animal material for research. All of the femurs and knee joints examined were from anatomically and radiologically normal knee joints, and were stored at −20°C until required. All age, bodyweight and gender data for the dogs used in these studies are shown in Table I.

ICN study

Measurements of the ICN were obtained from the femurs of cadaveric Golden Retrievers ($n = 23$), Labradors ($n = 26$) and Greyhounds ($n = 60$).

Impingement study

Ten Greyhound, ten Labrador Retriever and eight Golden Retriever knee joints were opened and placed in full extension. The areas of Impingement of the ICN on each ACL were marked with a permanent marker. The ACLs were removed from the joint by incising their tibial and femoral attachments and the marked impinged ligament section was removed.

ICN HEIGHT AND WIDTH MEASUREMENTS

Multiple measurements of the ICN were made as detailed by Fitch *et al.*[17] using digital Vernier callipers. The anterior, central and posterior notch widths (NW) and their indices (NWI), and the total condylar measurements were obtained

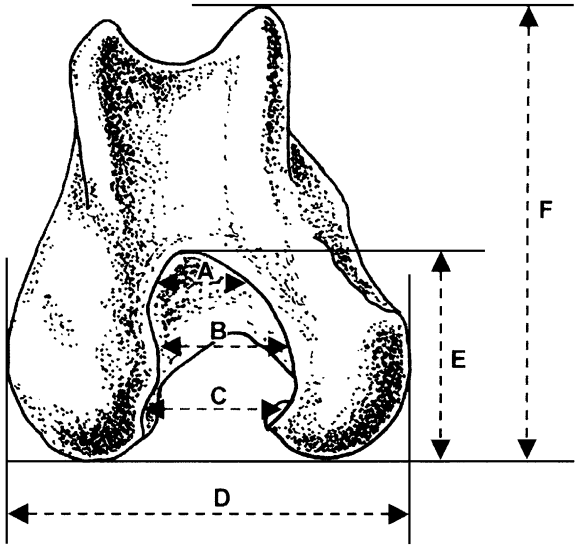

Fig. 1. Measurements of the canine ICN (adapted from Fitch *et al.*[17]) (A = anterior notch width, B = central notch width; C = posterior notch width, D = total condylar width, E = ICN height, F = femoral condyle height. The anterior NWI is A/D, the central NWI is B/D, and the posterior NWI is C/D, the NSI is B/E and ICN height index is E/F).

(Fig. 1). An estimation of the shape of the ICN was determined by the notch shape index (NSI), i.e., the central notch width divided by the ICN height[18].

BIOCHEMICAL ANALYSES IN ICN IMPINGEMENT STUDY

The impinged ligament sections (5–10 mg wet weight ligament) were analysed for total collagen content (by determination of hydroxyproline concentration), collagen cross-link type and content, matrix metalloproteinase (MMP) expression, activity and inhibition and sulphated and total glycosaminoglycan (GAG) concentrations using the standard methods previously described[19–23].

STATISTICAL ANALYSIS

All results are expressed as mean and standard error of mean (S.E.M.). Statistical tests were performed using statistical analysis software (Instat version 3.0, GraphPad Software, San Diego, USA). The dogs in this study, in both groups, were not matched for age, bodyweight or gender (Table I). However, multiple regression tests were performed for each of these background variables to investigate any relationships with age, bodyweight and gender. All of the ICN and biochemical parameters within the three groups were tested for normality and then compared using one way or

Table I
Age, gender and bodyweight data (mean ± S.E.M.) for the Greyhounds, Golden Retrievers and Labrador Retrievers in the ICN and impingement (IMP) study

Dog breed	Labrador Retriever ($n = 10$)	Golden Retriever ($n = 8$)	Greyhound ($n = 10$)	Labrador Retriever ($n = 26$)	Golden Retriever ($n = 23$)	Greyhound ($n = 60$)
ICN or IMP study	IMP	IMP	IMP	ICN	ICN	ICN
Age (years) (mean ± S.E.M.)	7.25 ± 1.1	7.1 ± 1.4	4.8 ± 0.5	7.0 ± 1.1	7.3 ± 0.9	4.4 ± 0.6
Weight (kg) (mean ± S.E.M.)	31.3 ± 1.9	31.5 ± 2.0	30.2 ± 1.2	29.1 ± 2.0	33.1 ± 1.7	29.4 ± 1.2
Gender (M and F)	M ($n = 5$)	M ($n = 7$)	M ($n = 5$)	M ($n = 11$)	M ($n = 21$)	M ($n = 34$)
	F ($n = 5$)	F ($n = 1$)	F ($n = 5$)	F ($n = 15$)	F ($n = 2$)	F ($n = 26$)

Table II
ICN dimensions and biochemical properties of impinged sections of ACLs (mean ± S.E.M.) from Greyhounds, Labrador and Golden Retrievers

ICN and biochemical measurements	Greyhounds	Labrador Retriever	Golden Retriever	*P* value
Femoral condylar width (mm)	35.9 (±0.7)	32.9 (±0.7)	31.3 (±0.5)	0.0001
Femoral condylar height (mm)	35.5 (±0.7)	30.6 (±0.9)	30.8 (±0.8)	0.0001
Anterior NWI (mm/mm)	0.13 (±0.01)	0.11 (±0.01)	0.10 (±0.01)	0.0008
Central NWI (mm/mm)	0.18 (±0.01)	0.16 (±0.01)	0.17 (±0.01)	0.019
Posterior NWI (mm/mm)	0.24 (±0.01)	0.22 (±0.01)	0.21 (±0.01)	0.045
Pro-MMP-2 (% standard)	28.2 (±6.2)	62.0 (±6.6)	49.0 (±10.0)	0.0025
actMMP-2 (% standard)	3.3 (±2.2)	36.5 (±7.2)	29.6 (±5.1)	0.0007
TIMP-2 (% standard)	15.0 (±2.3)	16.8 (±4.3)	34.8 (±5.0)	0.45
Collagen content (%)	47.47 (±7.8)	43.56 (±5.0)	53.2 (±7.3)	0.70
OH-Pyr (M/M collagen)	1.5 (±0.5)	0.6 (±0.4)	0.5 (±0.3)	0.0009
Sulfated GAGs (% weight)	0.4 (±0.1)	1.2 (±0.1)	1.2 (±0.2)	0.0008

two way analysis of variance test (Kruskal–Wallis test with post-test (Dunn's test) or Turkey–Kramer test, respectively) according to the result of the data normality test. All *P* values <0.05 were considered significant.

Results

ICN HEIGHT AND WIDTH INDICES

Femoral condyle width and height

The condylar width and height [Fig. 1(D and F)] are significantly greater in the Greyhounds compared to the Golden and Labrador Retrievers ($P < 0.001$ for both parameters). (Table II)

Width indices

The greatest difference among breeds was apparent in the anterior NWI [Fig. 2(a)], that of the Greyhounds being significantly larger than that of both the Labrador Retrievers ($P < 0.001$) and Golden Retrievers ($P < 0.01$). Although the difference in the central NWI was less pronounced, again that of the Greyhound was significantly different compared to the other two breeds ($P < 0.02$) [Fig. 2(b)]. The posterior NWI [Fig. 2(c)] was significantly different only among the Greyhounds compared with the Labrador Retrievers ($P < 0.05$).

Height indices

There was no significant difference in the ICN height index (ICN height/total condylar height) among the three breeds ($P = 0.11$).

NSI

No significant difference was found among any of the three breeds of dog ($P = 0.48$).

BIOCHEMICAL ANALYSES FOR ICN IMPINGEMENT STUDY

MMP-2 quantitation

The expression of pro MMP-2 [Fig. 3(a)] was significantly greater in the high risk breeds compared with the low risk breed ($P < 0.003$) (Table II). Similarly, the concentrations of active MMP-2 (actMMP-2) were significantly greater ($P < 0.001$) in both high risk breeds compared to the Greyhound [Fig. 3(b)]. The proportion of actMMP-2 relative to the pro MMP-2 was much higher in the high risk breeds, approximately 60% compared to 10%, in the low risk Greyhounds.

Tissue inhibitors of metalloproteinases-1 and -2 quantitation

There was no significant difference in the tissue inhibitors of metalloproteinases (TIMP)-2 concentrations among breeds ($P = 0.45$). TIMP-1 was not detected in these samples.

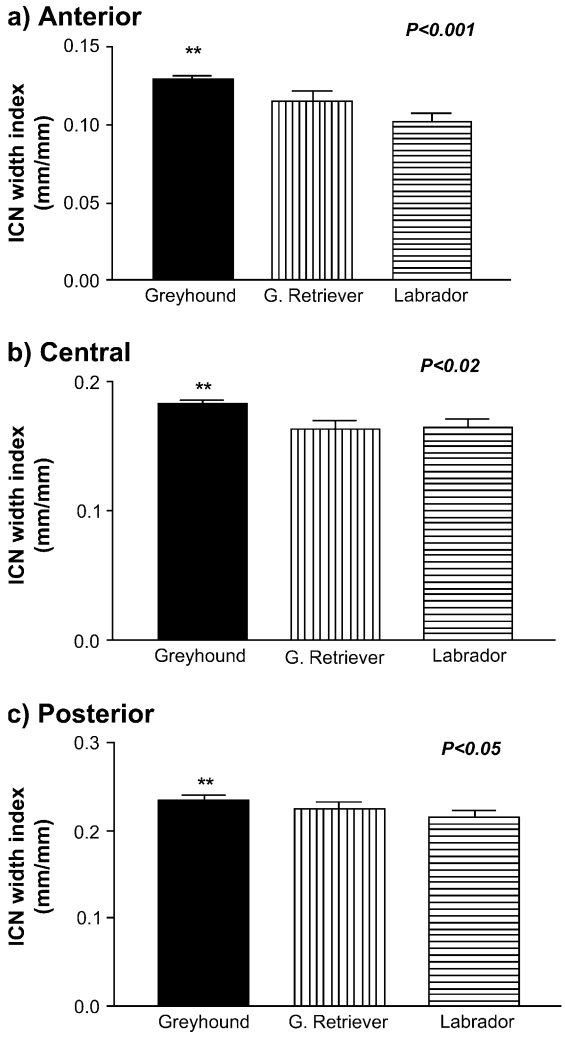

Fig. 2. ICN width indices (ICN width indices (anterior (a), central (b) and posterior (c)) in Greyhound ($n = 60$), Golden Retriever ($n = 23$) and Labrador Retriever ($n = 26$) femurs). (** denotes the stated statistical significance to the other two groups).

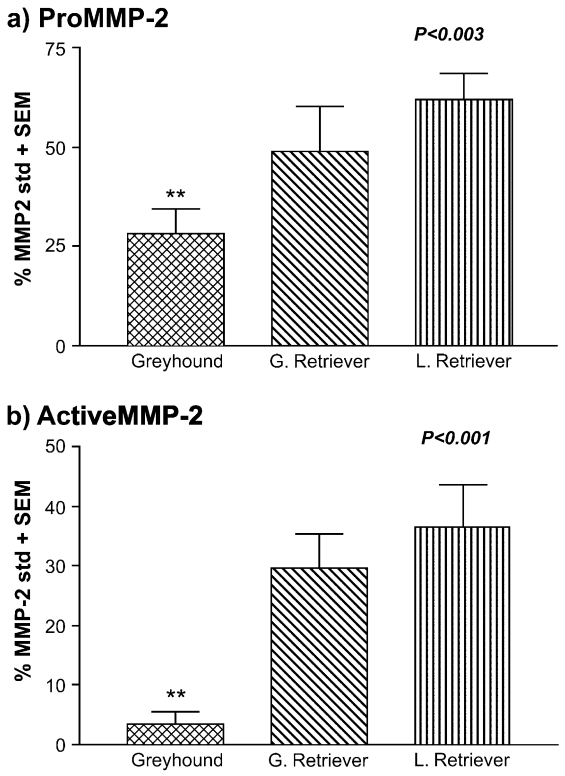

Fig. 3. The levels of pro (a) and active (b) MMP-2 (% standard) in the impinged parts of Greyhound ($n = 10$), Golden Retriever ($n = 8$) and Labrador Retriever ($n = 10$) ACLs. (** denotes the stated statistical significance to the other two groups).

Collagen content

There were not any significant differences in the total collagen content of the ACLs among the three breeds of dog ($P = 0.70$).

Collagen cross-links

Pyridinoline: The level of the predominant mature cross-link, hydroxylysyl-pyridinoline (OH-Pyr), in the Greyhound ACLs was greater ($P < 0.001$) compared with that in the Golden and Labrador Retrievers ACLs (Fig. 4).

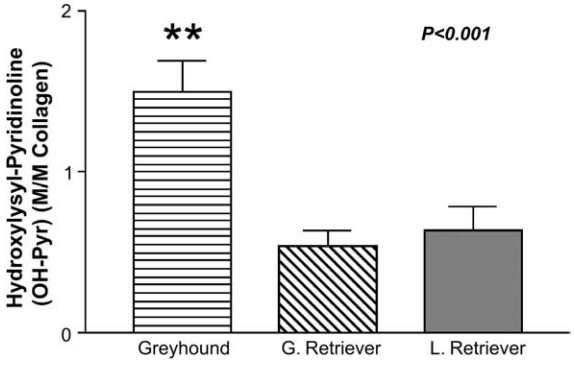

Fig. 4. The levels of the mature pyridinoline collagen cross-link (OH-Pyr) in the impinged parts of Greyhound ($n = 10$), Golden ($n = 8$) and Labrador Retrievers ($n = 10$) ACLs. (** denotes the stated statistical significance to the other two groups).

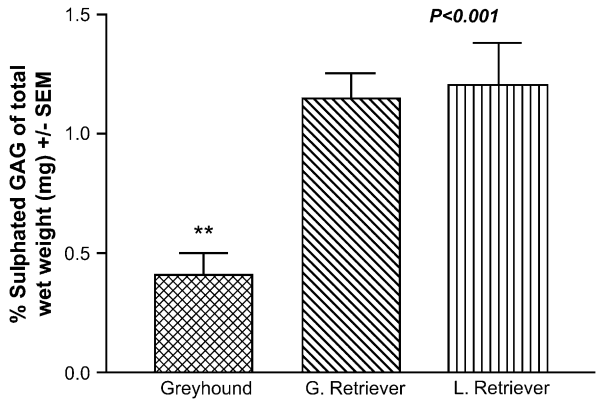

Fig. 5. The levels of sulphated GAG (% total wet weight) in the impinged parts of Greyhound ($n = 10$), Golden ($n = 8$) and Labrador Retrievers ($n = 10$) ACLs. (** denotes the stated statistical significance to the other two groups).

Dihydroxylysinorleucine: This intermediate cross-link was only demonstrated in Golden Retriever ACLs. *Hydroxylysinorleucine*: There were not any significant differences found among the three breeds for this intermediate cross-link ($P = 0.15$).

Sulphated GAGs: There were significantly more sulphated GAGs present in the rupture predisposed ACLs compared to the Greyhound ACLs ($P < 0.001$) (Fig. 5).

Total GAGs: There were not any significant differences seen among the three dog breeds ($P = 0.56$).

AGE, GENDER, BODYWEIGHT CORRELATIONS

Significant relationships among age, gender, and bodyweight and the ICN were only seen in the Labrador Retriever. The central NWI had a negative correlation with weight ($P = 0.004$, $r = 0.5$); as the bodyweight of the dog increased the NWI became smaller. Age had no significant relationship with any of the ICN measurements or indices in the three breeds. Age, gender and bodyweight did not correlate with the levels of sulphated GAGs, total GAGs, collagen content or collagen cross-link concentrations in these dogs.

Discussion

We have shown that the notch width index (NWI) of the breed at low risk for ACL rupture (Greyhound) is much larger than that of the high risk breeds (Golden and Labrador Retrievers). This may allow free movement of the ACL through the ICN (Fig. 2). In contrast, the NWI of the Golden and Labrador Retrievers are significantly narrower, resulting in the ACL impinging on the bone/cartilage structure, and under these conditions the ligament is subject to compressive as well as tensile stress.

Compression of T/L over bony surfaces is known to result in physiological adaptation to protect the tissue against the compressive force separating the collagen fibres. This is achieved by increasing the GAG content, resulting in an increase in water content and osmotic pressure creating a collagen network under pressure and ability to resist compression. These changes can lead to the formation of a "fibrocartilage" domain in the ligament or tendon by an up-regulation of the large proteoglycans, aggrecan and versican and type II collagen[10,24,25]. This is consistent with our finding of a significantly higher GAG content in the impinged

region of the high risk ACLs compared with that in the Greyhounds (Fig. 4).

This type of impingement of the ACL within the ICN has also been implicated in ligament and tendon failure in humans possibly due to the avascularity of the fibrocartilage regions[26–29]. However, the rupture of the ligaments or tendons does not always correspond to the fibrocartilage region[30]. Indeed, the nature of the fibrocartilage is highly variable, depending on the extent of the compressive forces, and its mechanical properties may therefore be correspondingly variable. Further, the differing biochemical composition between tensional and compressive regions of the ligament and how this influences the mechanical properties have not been investigated.

We have shown that the supporting collagenous structure is being remodelled, in the high risk ACLs, as a result of compression of the ligament, as evidenced by the higher expression and activity of the degradative enzyme MMP-2 and the lower concentration of the mature pyridinoline cross-link. Conversely, the Greyhound ACLs show little evidence of collagen turnover with low levels of MMP-2 expression and activation and high concentrations of the mature pyridinoline cross-link. The extracellular matrix (ECM) composition in rabbit ACLs has been shown to alter with abnormal joint biomechanics[31]. Similarly, increased knee joint laxity has been associated with altered ECM composition in ACLs from dogs with a differing predisposition to non-contact ACL rupture[32]. The replacement of mature collagen with newly synthesised collagen may lead to a reduction in the mechanical properties (e.g., ultimate tensile strength) of the ligament and ultimately to rupture. It may therefore be the remodelling of the ligament rather than the formation of the fibrocartilage, as suggested by other workers studying human cruciate ligaments, that causes degeneration and rupture.

Interestingly, the weight of the Labrador Retrievers was shown to be correlated with a decrease in the central NWI, whilst age did not correlate with any of the ICN parameters. It is possible that the increased load on the Labrador Retrievers knee joints alters the biomechanics of the joint and this may contribute to the ICN remodelling and narrowing. The bone around the ICN may be increasing due to adaptive remodelling, thereby narrowing the notch, possibly in response to the weakening of the ACL and reduced stability. Alternatively, the narrower notch width of the Labrador and Golden Retrievers may be developmental. A narrowed ICN may lead to ligament degeneration, the resultant instability may then be the cause of adaptive bone remodelling and further narrowing of the notch.

We have suggested in this study that the narrowed ICNs and resultant ACL impingement exert an influence on collagen turnover and the composition between high and low risk dog breeds to ACL disease. Alterations in ACL metabolism and composition secondary to the ICN narrowing may have an important role in the pathogenesis of ACL degeneration and ultimate rupture in dogs, leading to joint degeneration and OA. These findings may also have implications on our knowledge of the pathogenesis of this ACL injury in human medicine.

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